Nuclear Mutations in *Saccharomyces cerevisiae* That Affect the Escape of DNA from Mitochondria to the Nucleus

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ABSTRACT

We have inserted a yeast nuclear DNA fragment bearing the *TRP1* gene and its associated origin of DNA replication, *ARS1*, into the functional mitochondrial chromosome of a strain carrying a chromosomal *trp1* deletion. *TRP1* was not phenotypically expressed within the organelle. However, this Trp⁻ strain readily gave rise to respiratory competent Trp⁺ clones that contained the *TRP1/ARS1* fragment, associated with portions of mitochondrial DNA (mtDNA), replicating in their nuclei. Thus the Trp⁺ clones arose as a result of DNA escaping from mitochondria and migrating to the nucleus. We have isolated 21 nuclear mutants in which the rate of mtDNA escape is increased by screening for increased rates of papillation to Trp⁺. All 21 mutations were recessive and fell into six complementation groups, termed *YME1–YME6*. In addition to increasing the rate of mtDNA escape, *yme1* mutations also caused a heat-sensitive respiratory deficient phenotype at 37° and a cold-sensitive growth defect on complete glucose medium at 14°. While the other *yme* mutations had no detectable growth phenotypes, synergistic interactions were observed in two double mutant combinations: a *yme1, yme2* double mutant failed to respire at 30° and a *yme4, yme6* double mutant failed to respire at all temperatures tested. None of the respiratory defects were caused by loss of functional mtDNA. These findings suggest that *yme1*, *yme2*, *yme4* and *yme6* mutations alter mitochondrial functions and thereby lead to an increased rate of DNA escape from the organelle.

D URING the evolution of eucaryotes genetic information has apparently escaped from mitochondria and migrated to the nucleus. Direct observations in support of this notion include the presence of genes (NUGENT and PALMER 1991; VAN DEN BOOGAART, SAMALLO and AGSTERIBBE 1982), pseudogenes (FARRELLY and BUTOW 1983; GELLISSEN et al. 1983; JACOBS et al. 1983) and introns (LOUIS and HABER 1991) of apparent mitochondrial origin in the nuclear genomes of several different organisms. In addition, we have previously demonstrated the experimental detection of DNA escape from mitochondria to the nucleus in the yeast *Saccharomyces cerevisiae* (THORSNESS and FOX 1990). This was accomplished by introducing into mitochondria a plasmid bearing a genetic marker, *URA3*, that could be expressed only from the nucleus. Escape and migration of the plasmid DNA from mitochondria to the nucleus were monitored by observing the appearance of Ura⁺ cells expressing the genetic marker. The rate of these events was dependent upon the nuclear genetic background and a number of environmental factors, which included osmotic strength of the medium and incubation temperature during cell growth. However, while these experiments demonstrated that transfer of genetic information from mitochondria to the nucleus need not be a rare event, they shed little light on the

mechanism by which it occurs. For example, they did not distinguish whether the rate limiting step in the process was DNA escape from mitochondria or uptake of escaped DNA by the nucleus.

To begin a genetic analysis of the processes by which DNA moves from mitochondria to the nucleus we have now refined the detection system by introducing a nuclear DNA fragment bearing *TRP1* and *ARS1* into an otherwise unmodified fully functional yeast mitochondrial genome. Starting with a Trp⁻ strain bearing this novel mtDNA, we have isolated mutants in which the rate of mtDNA escape, monitored as the rate of appearance of Trp⁺ derivatives, is significantly increased.

The functions of the six nuclear genes (termed *YME* for yeast mitochondrial escape) so far identified by this novel genetic screen have not yet been determined, but our evidence indicates that at least some are related to mitochondria. It is well known that mutations in many yeast genes affecting mitochondrial respiratory functions prevent growth on nonrespiratory carbon sources [reviewed in PON and SCHATZ (1991) and TZAGOLOFF and DIECKMANN (1990)]. In addition, mutations that completely prevent the formation of mitochondria, because they block the import of proteins from the cytoplasm, prevent growth even on glucose-containing media [reviewed in BAKER

TABLE 1

Strains used in this study

Strain[a]	Nuclear genotype	Mitochondrial genotype[b]
PTH131ρ^0	*MATα; ura3-52; ade2-101; trp1-Δ1*	ρ^0
MCC123ρ^0	*MATa; ura3-52; ade2-101; kar1-1*	ρ^0
MCC123ρ^+, *TRP1*	*MATa; ura3-52; ade2-101; kar1-1*	ρ^+, *TRP1*
TF145	*MATα; ura3-d; ade2*	ρ^+, (*cox2-17*)
* PTY21ρ^0	*MATa, ura3-52; ade2; leu2-3, 112; trp1-Δ1*	ρ^0
PTY27	*MATα; ura3-52; ade2-101; trp1-Δ1*	ρ^-, *COX2* (pPT25)
* PTY28	*MATa; ade2; lys2; leu2-3, 112; trp1-Δ1*	ρ^+
PTY27XPTY28	*MATα; ura3-52; ade2-101; trp1-Δ1; LYS2; LEU2*	ρ^+, *TRP1*
	MATa; URA3; ade2; trp1-Δ1; lys2; leu2-3, 112	
* PTY29ρ^0	*MATα; lys2; leu2-3, 112; trp1-Δ1*	ρ^0
* PTY29ρ^+, *TRP1*	*MATα; lys2; leu2-3, 112; trp1-Δ1*	ρ^+, *TRP1*
* PTY30	*MATa; ura3-52, ade2; kar1-1*	ρ^-, *COX2* (pPT25)
* PTY33	*MATa; ura3-52; ade2; leu2-3, 112; trp1-Δ1*	ρ^+, *TRP1*
* PTY33ρ^0, Trp^{+c}	*MATa; ura3-52; ade2; leu2-3, 112; trp1-Δ1; TRP1 (ARS1)*	ρ^0
* PTY34	*MATa; ura3-52; lys2; leu2-3, 112; trp1-Δ1*	ρ^+, *TRP1*
* PTY43	*MATα; ura3-52; ade2; leu2-3, 112; trp1-Δ1*	ρ^+, *TRP1*
* PTY44	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1*	ρ^+, *TRP1*
* PTY53	*MATα; ura3-52; ade2; leu2-3, 112; trp1-Δ1; yme2-1; yme4-1*	ρ^+, *TRP1*
* PTY55	*MATα; ura3-52; ade2; leu2-3, 112; lys2; trp1-Δ1; yme1-1; yme2-1*	ρ^+, *TRP1*
* PTY62	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme1-1*	ρ^+, *TRP1*
* PTY64	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme2-1*	ρ^+, *TRP1*
* PTY66	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme3-1*	ρ^+, *TRP1*
* PTY68	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme4-1*	ρ^+, *TRP1*
* PTY70	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme5-1*	ρ^+, *TRP1*
* PTY72	*MATα; ura3-52; lys2; leu2-3, 112; trp1-Δ1; yme6-1*	ρ^+, *TRP1*

[a] Asterisk (*) indicates isogenic strains.
[b] All mitochondrial DNA sequences (excluding the *TRP1/ARS1* element) are derived from D273-10B mitochondrial DNA.
[c] There are 18 independent Trp$^+$ isolates of PTY33ρ^0, Trp$^+$. They differ only by the plasmid bearing the *TRP1/ARS1* element.

and SCHATZ (1991)]. Several mutations in one of the genes we have identified, *YME1*, cause both heat sensitivity of growth on nonrespiratory carbon sources and cold sensitivity of growth on complete glucose medium, in addition to increasing the rate at which mtDNA moves to the nucleus. Thus, this gene, at least, appears to specify an important mitochondrial function.

MATERIALS AND METHODS

Strains, strain constructions and genetic methods: The *Escherichia coli* strain used for preparation and manipulation of DNA was DH5α [F-, *endA1, hsdR17(rk-mk+), supE44, thi-1, λ, recA, gyrA96, relA1, Δ(argF-laczya), U169, ϕ80 lac ZΔM15*]. The *E. coli* strain CJ236 [*dut, ung, thi, relA*; pCJ105 (Cm^r)] was used for the generation of uridine labeled single-stranded DNA.

The genotypes for the *S. cerevisiae* strains used in this work are listed in Table 1. Standard genetic techniques were used to construct and analyze the various yeast strains (SHERMAN, FINK and HICKS 1986) except as noted below. PTH231ρ^0 was transformed with plasmid pPT25 by microprojectile bombardment (FOX, SANFORD and MCMULLIN 1988; JOHNSTON *et al.* 1988) in a manner previously described (FOX *et al.* 1990; THORSNESS and FOX 1990). A yeast strain transformed with the plasmid pPT25 in both mitochondria and the nucleus was cultured on complete media, and a Trp$^-$, ρ^- segregant isolated and named PTY27. This strain was mated to PTY28 and a diploid selected that

contained a recombined mitochondrial genome containing the *TRP1* gene (see RESULTS). In order to move the ρ^+, *TRP1* mitochondrial genome into a defined nuclear background, the diploid PTY27XPTY28 was sporulated and a spore mated to the *kar1-1* strain, MCC123ρ^0. A segregant was isolated containing the MCC123 nucleus and the ρ^+, *TRP1* mitochondria and designated MCC123ρ^+, *TRP1*. ρ^+, *TRP1* mitochondria were in turn introduced into a strain isogenic to D273-10B, PTY29ρ^0, via a cross with MCC123ρ^+, *TRP1* to give rise to PTY29ρ^+, *TRP1*. The isogenic strains PTY21ρ^0 and PTY29ρ^+, *TRP1* were mated, sporulated, and tetrads dissected to give rise to the haploid strains PTY33, PTY43 and PTY44. In order to generate an isogenic strain containing the ρ^-[*TRP1/ARS1/COX2*] mitochondria of PTY27, the *kar1-1* strain MCC123ρ^0 was used to passage mitochondria into PTY29ρ^0 to give PTY39 which is isogenic to D273-10B. ρ^0 derivatives of phenotypically Trp$^+$ isolates of PTY33 (see Results) were made by culturing in minimal media containing 25 μg/ml ethidium bromide as previously described (FOX *et al.* 1990). The double *yme* mutant strains PTY53 and PTY55 were generated by crossing a *yme4-1* strain with a *yme6-1* strain and a *yme1-1* strain with a *yme2-1* strain, respectively. Diploids were sporulated, and spores analyzed for escape phenotype and growth characteristics on YPD and YPEG.

Media: *E. coli* containing plasmids were grown in LB (10 g Bacto-tryptone, 10 g NaCl, 5 g yeast extract per liter) plus 125 μg/ml ampicillin. Yeast were grown in YPD, YPEG or SD + nutrients. One liter of YPD contained 20 g glucose, 20 g Bacto-peptone, 10 g yeast extract and was supplemented with 40 mg tryptophan. One liter of YPEG contained 30 ml glycerol, 30 ml ethanol, 20 g Bacto-peptone,

10 g yeast extract and was supplemented with 40 mg tryptophan. One liter of SD + nutrients contained 6.7 g yeast nitrogen base without amino acids, 20 g glucose and the appropriate nutrients for the experiment. Nutrients were uracil at 40 mg/liter, adenine at 40 mg/liter, tryptophan at 40 mg/liter, lysine at 60 mg/liter and leucine at 60 mg/liter. For agar plates, Bacto-agar was added at 20 g/liter. One liter of sporulation media contained 10 g potassium acetate, 1 g yeast extract, 0.5 g glucose and 20 g Bacto-agar. Bacto-agar, Bacto-peptone, Bacto-tryptone, yeast extract and yeast nitrogen base without amino acids were obtained from Difco. Ampicillin and nutrients were obtained from Sigma.

Nucleic acid techniques: Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratories and New England Biolabs. Standard techniques for generating recombinant DNAs and performing DNA blot hybridizations were used (MANIATIS, FRITSCH and SAMBROOK 1982). Site directed mutagenesis was accomplished with the aid of the Bio-Rad Mutagene Kit.

Construction of plasmids: A 2.5-kb EcoRI/HindIII fragment containing the COX2 gene was excised from pMK2 (THORSNESS and FOX 1990). This fragment was ligated into the EcoRI and HindIII sites in the polylinker of pBLUESCRIPT (KS, M13+) generating pPT21. The EcoRI site of pPT21 was destroyed by digestion with EcoRI followed by treatment with the Klenow fragment of DNA polymerase. Single stranded DNA was made from this plasmid, pPT22, using the dut, ung strain CJ236. Using the Mutagene Kit from Bio-Rad and an oligonucleotide of the sequence (mismatch is underlined):

5'-CACTCCTTACGGAATTCCCGCTTCC-3'

a single base was changed in the mtDNA sequence approximately 285 base pairs upstream of the translational start of the COX2 gene. In the resulting plasmid, pPT24, there are approximately 200 base pairs of authentic mtDNA 5' of the introduced EcoRI site, and 2.3 kb of mtDNA containing the COX2 gene 3' of the introduced EcoRI site. pPT24 was then digested with EcoRI and a 1.5-kb EcoRI fragment containing the TRP1 gene and ARSI element from YRp7 inserted, generating pPT25. The reading frames of TRP1 and COX2 were oriented in the same direction in pPT25.

Isolation of escape mutants: Yeast strain PTY43 was mutagenized with ethyl methanesulfonate as described by LAWRENCE (1990). The mutagenized cells were plated on SD + adenine + uracil + leucine + tryptophan at a density such that one cell gave rise to a distinct single colony. The plates were incubated at 30° for 5 days and then replica plated to SD + adenine + uracil + leucine. These plates were incubated for 5 days, with a daily scoring for papillation to Trp+ growth. Those colonies showing heavy papillation to Trp+ growth compared to the unmutagenized control were colony purified on YPEG and rescreened for an increased rate of DNA escape phenotype on SD + adenine + uracil + leucine. After several rounds of colony purification and rescreening, during which a number of putative mutant strains were eliminated, the mutant cells were backcrossed to PTY34 and the diploids were scored for the escape phenotype. The diploids were also sporulated and the tetrads dissected. Each colony arising from a spore was scored for nutritional markers, mating type and the rate at which DNA escaped and migrated from mitochondria to the nucleus.

RESULTS

Construction of a ρ+ mitochondrial chromosome carrying TRP1: Much of the previous work demonstrating the escape of DNA from mitochondria to the nucleus was done with a nonrespiring "synthetic" ρ⁻ strain whose mitochondria contained the URA3 gene on a plasmid (THORSNESS and FOX 1990). Genetic analysis of nonrespiring yeast strains is cumbersome because they are unable to sporulate. To continue these studies we therefore constructed strains carrying a functional ρ+ mitochondrial genome in which a selectable nuclear gene and a closely associated nuclear origin of replication were inserted. A plasmid bearing the mitochondrial gene COX2 was subjected to oligonucleotide-directed mutagenesis to create a unique EcoRI site approximately 285 base pairs upstream of the COX2 translational start site. This region of mtDNA has no known function. A 1.5-kb EcoRI fragment encoding the TRP1 gene and the nuclear origin of replication ARS1 was inserted at the new EcoRI site upstream of COX2 to create the plasmid pPT25.

Plasmid pPT25 is capable of replication in both the nucleus and mitochondria. The ρ⁰, trp1 yeast strain PTH231ρ⁰ was transformed with pPT25 by microprojectile bombardment (Fox et al. 1990). Nuclear transformants were identified by complementation of the trp1-Δ1 mutation by the plasmid borne TRP1. Mitochondrial transformants were identified among the nuclear transformants based upon their ability to produce respiring diploids when mated to a tester strain bearing a COX2 deletion mutation. One such mitochondrial transformant was grown on complete medium, after which a Trp⁻ clone, no longer containing pPT25 in the nucleus, was identified and isolated. (Trp⁻ auxotrophs retaining the plasmid in their mitochondria could be readily isolated since the ARS1 origin does not promote efficient transmission of plasmid DNA during mitotic growth.) This Trp⁻, ρ⁻ strain, PTY27, was then mated to a trp1, ρ+ strain, PTY28. Zygotes were plated on medium that selected for diploids at a density such that single colonies could be distinguished. These diploid colonies were then replica plated to media that lacked tryptophan. We reasoned that diploid clones whose mtDNA contained the TRP1 gene, as a result of homologous recombination (diagrammed in Figure 1), would be able to yield Trp+ cells as a result of escape of mtDNA to the nucleus. Trp+ cells from the edges of replica plated diploid colonies were streaked on YPEG plates (selecting for respiratory growth, but not for maintenance of TRP1 in the nucleus), and then rescreened for their ability to give rise to Trp+ cells. Several isolates were found that gave rise to Trp+ cells from a population of Trp⁻ cells. The Trp+ character of these cells was mitotically unstable, as expected for an ARS plasmid in the nucleus. The mtDNA from these diploid strains was examined by DNA blot hybridization analysis (data not shown) and found to have the
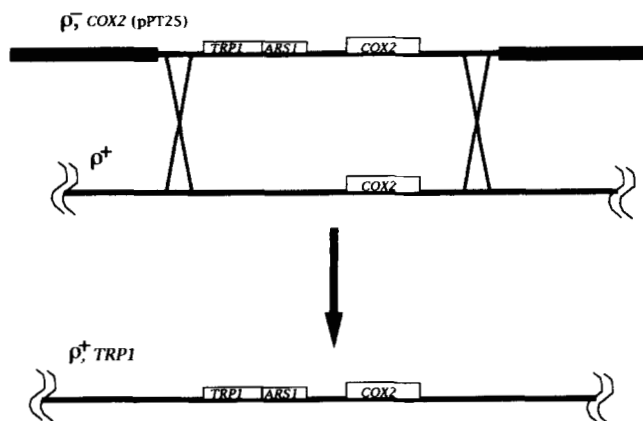
FIGURE 1.—Integration of a nuclear gene into the mitochondrial genome. The top line corresponds to the plasmid pPT25 (the thin lines indicate mtDNA, the thick lines indicate bacterial DNA), present in the mitochondria of strain PTY27. PTY27 was mated with the wild-type ρ^+ strain PTY28. The depicted homologous double recombination event gave rise to rise to the ρ^+, *TRP1* mitochondrial chromosome.

TABLE 2

Relative rates of DNA escape from mitochondria

Strain	Relevant nuclear genotype	Mitochondrial genotype	Relative rate of DNA escape
PTY39	Wild type	ρ^- (*TRP1, COX2*)	32
PTY44	Wild type	ρ^+, *TRP1*	1
PTY62	*yme1-1*	ρ^+, *TRP1*	25
PTY64	*yme2-1*	ρ^+, *TRP1*	8
PTY66	*yme3-1*	ρ^+, *TRP1*	10
PTY68	*yme4-1*	ρ^+, *TRP1*	11
PTY70	*yme5-1*	ρ^+, *TRP1*	6
PTY72	*yme6-1*	ρ^+, *TRP1*	12

The rate of escape and migration of DNA from mitochondria to the nucleus was determined numerically using an equation derived by LURIA and DELBRUÜCK (1943), as described previously (THORSNESS and FOX 1990). For each strain, single colonies that had been grown on YPEG (for ρ^+, *TRP1* strains) or on YPD (for PTY39) for two to three days were excised and suspended in water. Aliquots were plated on YPD to determine the total number of cells and on SD + adenine + uracil + leucine + lysine to determine the number of Trp$^+$ cells in each colony. The rate of DNA escape from mitochondria and the subsequent migration to the nucleus for each strain was normalized to the rate for PTY44. The true rate for each strain (events/cell/cell division) is the relative rate $\times 5 \times 10^{-6}$.

structure shown in Figure 1. We have designated this altered mitochondrial chromosome as "ρ^+, *TRP1*."

Characterization of strains bearing a ρ^+, *TRP1* mitochondrial chromosome: Diploid cells containing the ρ^+, *TRP1* mtDNA were fully capable of respiratory growth on nonfermentable carbon sources and of sporulation. The ρ^+, *TRP1* mitochondria were moved into the standard D273-10B nuclear genetic background by cytoduction using *kar1-1* mutant strains. The rate of DNA escape from mitochondria to the nucleus in this genetic background was measured by a statistical fluctuation analysis. As shown in Table 2, DNA escaped and migrated from mitochondria to the

nucleus in a respiring ρ^+ strain at a 30-fold slower rate than from the isogenic ρ^- strain.

The structure of the mtDNA that escaped from mitochondria and migrated to the nucleus was investigated by DNA blot hybridization analysis. Eighteen independent spontaneous Trp$^+$ isolates from strain PTY33 were selected and colony purified on media that selected for tryptophan prototrophs. The mtDNA in these strains was eliminated by treatment with ethidium bromide (MATERIALS AND METHODS). (Conversion to ρ^0 was verified by mating to a *COX2* deletion strain, TF145: none of the resulting diploids could respire.) As expected, the resulting ρ^0 derivatives still exhibited a mitotically unstable Trp$^+$ phenotype. Total DNA was prepared from the Trp$^+$ PTY33ρ^0 isolates and digested with the restriction enzyme *Bgl*II. *Bgl*II was chosen for the initial screening because it cuts within the *TRP1/ARS1* fragment but infrequently in mtDNA. The restricted DNA was separated on an agarose gel, transferred to a nitrocellulose membrane and the membrane probed with ^{32}P-labeled total mtDNA. The autoradiogram of the probed membrane is shown in Figure 2a. The sizes of the escaped mtDNAs bearing the *TRP1* gene that were replicating as plasmids in the nucleus ranged from approximately 7 kb to greater than 30 kb. Further analysis of the PTY33ρ^0 Trp$^+$ isolates demonstrated that the *TRP1/ARS1* element had been accompanied to the nucleus by varying amounts of flanking mtDNA in the various isolates (data not shown). A map of a representative plasmid, composed of escaped mtDNA that was found in the nucleus of one isolate, is shown in Figure 2b. The recombination site that led to circularization of this DNA has not been pinpointed.

Previous work had shown that the escape of DNA from ρ^- cells was influenced by both environmental and genetic factors (THORSNESS and FOX 1990). Before attempting to isolate mutations that affected the rate of DNA escape from mitochondria we wished to determine whether a nuclear mutation blocking respiration would influence the rate of DNA escape from mitochondria. We therefore crossed a ρ^0 strain carrying a *pet54* mutation with the ρ^+, *TRP1* strain. *PET54* encodes a protein required for the translation of *COX3* messenger RNA in mitochondria (COSTANZO, SEAVER and FOX 1986, 1989). Analysis of the meiotic progeny of this cross revealed no significant difference in the rate of DNA escape from mitochondria between Pet$^+$ and Pet$^-$ strains (data not shown).

Isolation of mutations in nuclear genes that affect the rate of DNA escape and migration from mitochondria to the nucleus: A mutant screen designed to isolate mutations affecting the rate of DNA escape from mitochondria was based on identifying strains of yeast that produced an increased number of Trp$^+$
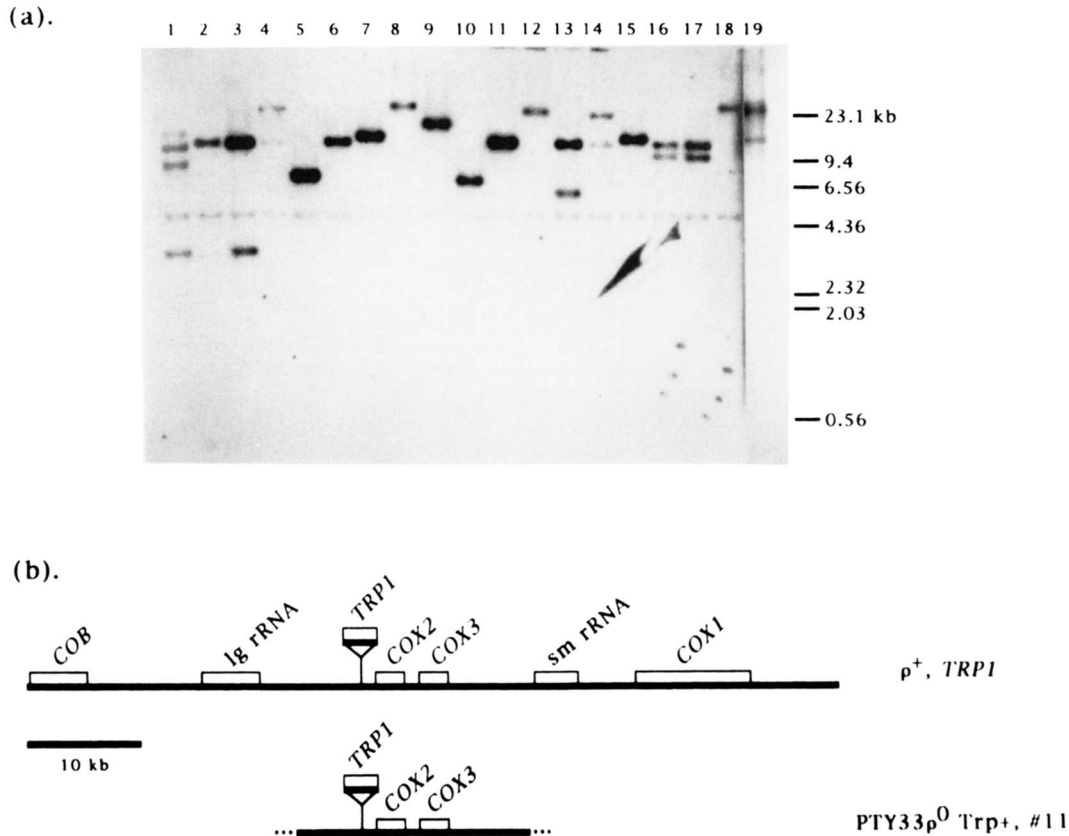
(a).

(b).

FIGURE 2.—DNA blot hybridization analysis of DNA molecules in the nucleus derived from mtDNA. (a) Eighteen independent Trp⁺ clones were isolated from strain PTY33 and subsequently treated with ethidium bromide to remove mtDNA (MATERIALS AND METHODS). Total DNA was prepared from these 18 independent ρ^0, Trp⁺ isolates (MATERIALS AND METHODS). The DNA was digested with *Bgl*II, electrophoresed on a 1% agarose gel, transferred to nitrocellulose, and probed with ³²P-labeled wild-type mtDNA. There is a single *Bgl*II site in the *TRP1/ARS1* fragment and three *Bgl*II sites in wildype ρ^+ DNA (DUJON 1981; GRIVELL 1987). The faint band at 4.4 kb in all of the lanes is the result of a preexisting nuclear DNA sequence. Lanes 1–18, independent isolates of PTY33ρ^0, Trp⁺ #1 through #18; lane 19, PTY33. Lanes 1–18 are from a 93-hr exposure and lane 19 is from a 4.5-hr exposure. (b) To partially characterize the extent of mtDNA sequences present in the nucleus of strain PTY33ρ^0, Trp⁺ #11, its DNA was further analyzed by restriction digestion and DNA blot hybridization. Comparison with the circular map of wild-type ρ^+ DNA (DUJON 1981; GRIVELL 1987), arbitrarily linearized here, is consistent with the map of contiguous mtDNA sequences shown for PTY33ρ^0, Trp⁺ #11. Since linear DNA molecules cannot replicate in yeast nuclei without telomeres (SZOSTAK and BLACKBURN 1982), we assume that the mtDNA bearing *TRP1/ARS1* is circular. The points in the mtDNA sequence where circularization occurred by recombination have not been determined precisely and this uncertainty is indicated by the dots at the ends of the PTY33ρ^0, Trp⁺ #11 map.

papillae on media lacking tryptophan. Colonies producing large numbers of papillae could arise either as a result of a single escape event that occurred early during the growth of the colony, or an increased number of escape events during the growth of the colony. These two possibilities could be easily distinguished by restreaking to test the heritability of the trait.

Mutagenized cells were plated on minimal glucose media at a density such that single cells gave rise to distinct colonies. These colonies were then replica plated to minimal glucose media lacking tryptophan and incubated at 30°. Colonies that gave rise to unusually large numbers of Trp⁺ papillae were streaked on nonfermentable YPEG medium and rescreened for Trp⁺ papillation several times to identify stable mutants. We demanded that all putative mutants respire since we had observed that a ρ^- strain had a 30-

fold greater rate of DNA escape from mitochondria than an isogenic ρ^+ strain (Table 2). From a total of roughly 10,000 mutagenized clones, we identified 21 mutant strains exhibiting an increased rate of escape and migration of DNA from mitochondria to the nucleus.

These mutant yeast strains were backcrossed to an isogenic wild-type strain. All of the mutations that led to an increase in the rate of DNA escape were recessive. The diploids were sporulated, and tetrads were scored for the rate of DNA escape from mitochondria. In all cases the escape phenotype segregated 2:2, indicating single mutations in nuclear DNA. Complementation was studied by crossing strains bearing all 21 mutations with each other. Diploids were selected, replica plated to minimal media lacking tryptophan and the rate and extent of papillae formation was scored. Those heterozygous diploids exhibiting rates

TABLE 3

Mitochondrial DNA escape mutants

| Loci	No. of alleles	Collateral phenotypes[a]
yme1	6	Pet⁻ at 37°; no growth on rich glucose media at 14°
yme2	3	*yme1, yme2* double mutant is Pet⁻ at 30° and 37°
yme3	9	
yme4	1	*yme4, yme6* double mutant is Pet⁻ at 14°, 30° and 37°
yme5	1	
yme6	1	*yme4, yme6* double mutant is Pet⁻ at 14°, 30° and 37°

[a] Single mutants and all possible pairwise double mutant strains were examined for growth on YPD and YPEG at 14°, 30°, and 37°. The inability to carry out respiratory growth on YPEG is designated by "Pet⁻." No collateral phenotypes involving the *yme3* or *yme5* mutant strains were observed.
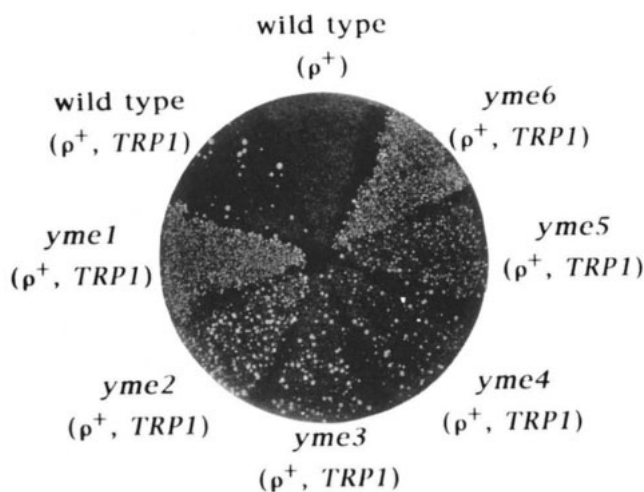
FIGURE 3.—Replica assay for the escape and migration of DNA from mitochondria to the nucleus. The strains bearing the genetic markers indicated were heavily streaked on a YPEG plate and grown for 3 days at 30°. They were then replica plated to SD + adenine + uracil + leucine + lysine and incubated at 30° for five days. Strains: wild-type (ρ⁺), PTY28; wild-type (ρ⁺, *TRP1*), PTY44; *yme1* (ρ⁺, *TRP1*), PTY62; *yme2* (ρ⁺, *TRP1*), PTY64; *yme3* (ρ⁺, *TRP1*), PTY66; *yme4* (ρ⁺, *TRP1*), PTY68; *yme5* (ρ⁺, *TRP1*), PTY70; *yme6* (ρ⁺, *TRP1*), PTY72.

of mtDNA escape that were qualitatively equivalent to the relevant homozygous diploids were judged to contain noncomplementing mutations. The 21 mutations fell into six complementation groups (Table 3). The rate of DNA escape and migration from mitochondria to the nucleus was measured for a representative member of each complementation group as shown in Table 2. The replica plate assay for the mutants, the isogenic wild-type ρ⁺, *TRP1*, an isogenic wild-type ρ⁺, and an isogenic ρ⁻ (*TRP1/ARS1*) is shown in Figure 3. The genetic loci identified by these mutations have been designated *YME1* through *YME6* (for *y*east *m*itochondrial *e*scape).

Despite the fact that all of the mutants were selected
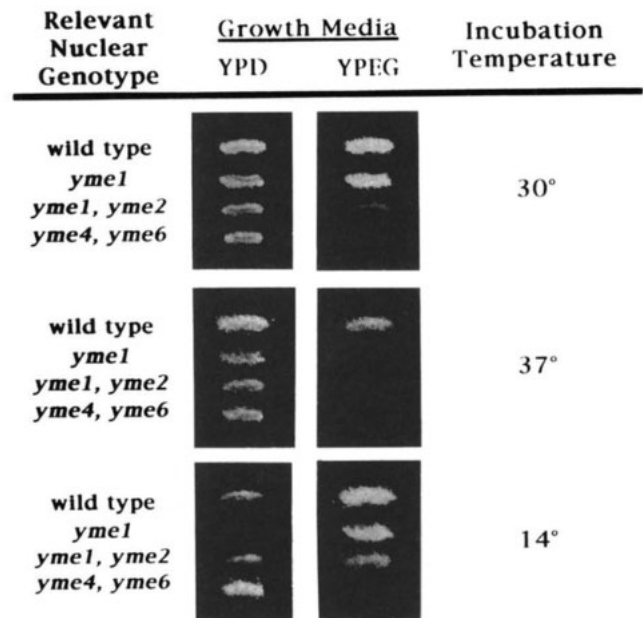
FIGURE 4.—Growth sensitivities of the *yme1-1*, the *yme1-1/yme2-1* and the *yme4-1/yme6-1* mutant strains. Wild-type and mutant strains growing on YPD were replica plated to YPD and YPEG media and incubated at the indicated temperatures. Strains: wild type, PTY44; *yme1-1*, PTY62; *yme1,yme2*, PTY55; *yme4, yme6*, PTY53.

to grow on nonfermentable medium, it was possible that the increased rate of mtDNA escape was due to an increased rate of ρ⁻ formation. We therefore measured the fraction of ρ⁻ cells in glucose-grown cultures of strains carrying a representative mutation from each *yme* complementation group. No mutant strain accumulated more than twice the fraction of ρ⁻ cells observed in the wild-type culture (0.9%). We thus conclude that elevation of the frequency of ρ⁻ formation is not the mechanism by which the *yme* mutations affect the rate of mtDNA escape.

Two collateral phenotypes were associated with mutations in the *YME1* complementation group. *yme1* mutants were unable to grow on YPEG at 37° but were able to grow on YPEG at 30° (Figure 4). This heat-sensitive respiratory defect was tightly linked to the *yme1* locus as identified by the phenotype of high rate of DNA escape from mitochondria: 18 out of 18 complete tetrads from a cross to wild type were parental ditypes for the temperature sensitivity and high rate of DNA escape phenotypes. The second phenotype associated with *yme1* mutant strains was the inability to grow on rich glucose medium, YPD at 14° (Figure 4). Interestingly, however, *yme1* mutants can grow at 14° on the nonfermentable medium YPEG (Figure 4), and on minimal glucose medium (not shown).

Two combinations of *yme* mutations were found to interact synergistically, producing synthetic respiratory defective phenotypes. Both a *yme1-1, yme2-1* double mutant strain and a *yme4-1, yme6-1* double mutant

strain were unable to grow on YPEG at either 30° or 37°, and the *yme4-1, yme6-1* double mutant strain was also unable to respire at 14° (Figure 4). Surprisingly, the *yme2-1* mutation partially suppressed the cold-sensitive growth phenotype caused by *yme1-1* on YPD medium. Both the *yme1-1* mutant, grown at the restrictive temperature, and the *yme4-1, yme6-1* and the *yme1-1, yme2-1* double mutants retained mtDNA as evidenced by their ability to complement the respiratory defect of a ρ^0 tester strain (not shown). Thus, mutations in four of the six *yme* complementation groups appear to affect mitochondrial physiology in addition to causing elevated rates of mtDNA escape.

DISCUSSION

We have developed a novel screen for yeast mutants in which the rate of DNA movement from mitochondria to the nucleus (THORSNESS and FOX 1990) is increased. The purpose of this study was to begin examining the mechanism by which this intracellular transfer of genetic information takes place and thereby, perhaps, to explore new facets of mitochondrial biology.

Our genetic assay depended on the construction of a yeast strain in which a defined fragment of nuclear DNA, carrying the nuclear genetic marker *TRP1* and its associated origin of nuclear DNA replication *ARS1*, was imbedded in a fully functional mitochondrial chromosome. The mitochondrially located *TRP1* gene was not phenotypically expressed, as expected. However, Trp⁺ clones could be readily isolated from such a strain. These clones were found to have the *TRP1/ARS1* fragment, associated with portions of mtDNA, replicating in their nuclei. These Trp⁺ clones could still respire and thus retained copies of functional mtDNA. The assay, therefore, detects the end-product of a series of events: escape of some (but not all) DNA from mitochondria, localization of that DNA to the nucleus, replication of that DNA in the nucleus, and phenotypic expression of the nuclear gene.

We screened for mutants in which the rate of appearance of Trp⁺ clones was higher than that of the starting wild-type strain. Twenty one independent mutants were isolated. All contained recessive nuclear mutations that fell into six complementation groups, termed *YME1–YME6*. We do not yet know which steps in the pathway leading to Trp⁺ clones are affected by the mutations. In addition to the rate of escape of DNA from mitochondria, the mutations could alter the efficiency of localization of DNA to the nucleus, replication and/or segregation of the escaped DNA in the nucleus, or even expression of the *TRP1* gene itself.

At present, the best argument that at least some of the *YME* genes specify mitochondrial functions is the fact that *yme1* mutations prevent respiratory growth

at elevated temperature, in addition to increasing the measured frequency of intracellular DNA movement. Furthermore, although single mutations at the other *YME* genes did not cause detectable defects in respiratory growth, two double mutant combinations produced synthetic respiratory phenotypes. A *yme4-1, yme6-1* double mutant failed to grow on nonfermentable medium at all temperatures tested, and the heat-sensitive respiratory defect caused by the *yme1-1* mutation was exacerbated by the presence of the *yme2-1* mutation in the same haploid nucleus. In none of these cases were the respiratory defects caused by loss of functional mtDNA. These findings suggest that the *yme1, yme2, yme4* and *yme6* mutations alter mitochondrial functions that lead directly to an increased rate of DNA escape from the organelle. It is important to note that respiratory deficiency *per se*, caused by a *pet54* mutation (COSTANZO, SEAVER and FOX 1986, 1989), did not increase the rate of DNA escape.

There are a number of possible mechanisms by which DNA might escape from mitochondria. For example, there may be transient breaches of the inner mitochondrial membrane. These could occur either spontaneously or during organellar division and/or fusion. Another reason for DNA escape might be the occasional incomplete destruction of mtDNA during terminal degradation of a mitochondrion. Thus one could imagine that *yme* mutations might impair the membrane integrity of mitochondria by subtly affecting membrane composition, organellar division and/or fusion, or organellar degradation.

In addition to causing heat sensitivity of respiratory growth, the *yme1-1* mutation also prevents growth at low temperatures on complete medium containing the fermentable carbon source glucose. This cold-sensitive phenotype is also consistent with a possible mitochondrial function for the *YME1* gene product. Mitochondria contain enzymes necessary for numerous vital metabolic functions in addition to oxidative phosphorylation, and intact mitochondrial compartments are essential for viability even on fermentable carbon sources (BAKER and SCHATZ 1991). Thus *YME1* function may be necessary for formation, maintenance or propagation of mitochondrial organelles in the presence of complete glucose medium at low temperature. However, *YME1* function is not absolutely required for growth at low temperatures. The *yme1-1* cold-sensitive phenotype is partially suppressed by the *yme2-1* mutation. Furthermore, *yme1-1* does not prevent growth at low temperature on either minimal glucose medium or medium containing nonfermentable carbon sources. These results could suggest that there may be other *YME1*-like genes in yeast whose expression is repressed during growth on complete glucose media. An alternative explanation might be that *yme1* mutations slow a critical process at low temperature

that blocks vigorous cell growth on rich media. In this model, inherently slower growth on poorer media would allow cells to bypass the block. One precedent for such a model is provided by the suppression of certain growth and secretion defects, caused by certain *secA* mutations of *E. coli*, by both mutations and drugs that slow the rate of protein synthesis (LEE and BECKWITH 1986).

Insertion of nuclear DNA sequences into a functional yeast mitochondrial chromosome significantly extends the demonstrated range of directed alterations of the mitochondrial genome by transformation and homologous recombination (ANZIANO and BUTOW 1991; FOLLEY and FOX 1991; FOX *et al.* 1990). It should be possible to insert virtually any sequence into yeast mtDNA. Furthermore, since the presence of yeast nuclear genes in mtDNA can be detected by virtue of their ability to escape to the nucleus, it should be possible to use such sequences as genetic markers on the mitochondrial chromosome despite the fact that they are not phenotypically expressed within the organelle.

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